

## Crystal Structure of Mammalian Poly(A) Polymerase

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Beamline(s): X12C

**Introduction:** In eukaryotes, polyadenylation of pre-messenger RNA plays an essential role in the initiation step of protein synthesis, as well as in the export and stability of mRNAs. Poly(A) polymerase (PAP), the enzyme at the heart of the polyadenylation machinery, is a template-independent RNA polymerase that specifically incorporates ATP at the 3'-end of mRNA.

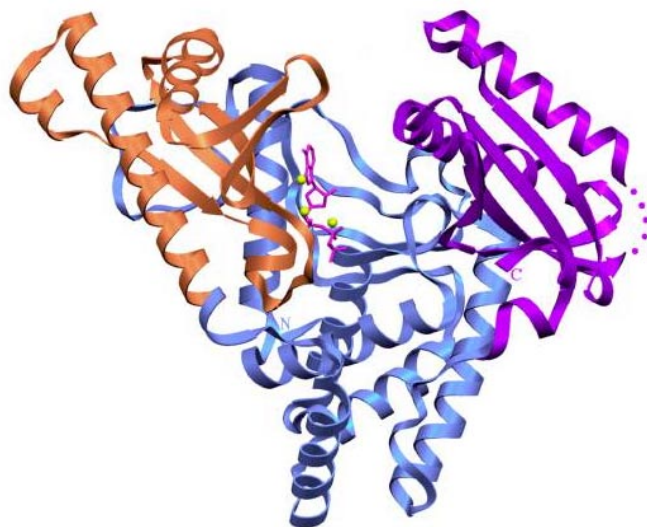
**Methods and Materials:** We have engineered selenomethionyl-PAP. We collected a three wavelength MAD data set on selenomethionyl PAP crystals at X12C in October 1999.

**Results:** We have solved the crystal structure of bovine poly(A) polymerase bound to an ATP analog at 2.5 Å resolution (Martin *et al.*, 2000). The structure revealed expected and unexpected similarities to other proteins. As expected, the catalytic domain of poly(A) polymerase shares substantial structural homology with other nucleotidyl transferases such as DNA polymerase and kanamycin transferase. The C-terminal domain unexpectedly folds into a compact domain reminiscent of the RNA recognition motif fold. The three invariant aspartates of the catalytic triad ligate two of the three active site metals. One of these metals also contacts the adenine ring. Furthermore, conserved, catalytically important residues contact the nucleotide. These contacts, taken together with metal coordination of the adenine base, provide a structural basis for ATP selection by poly(A) polymerase.

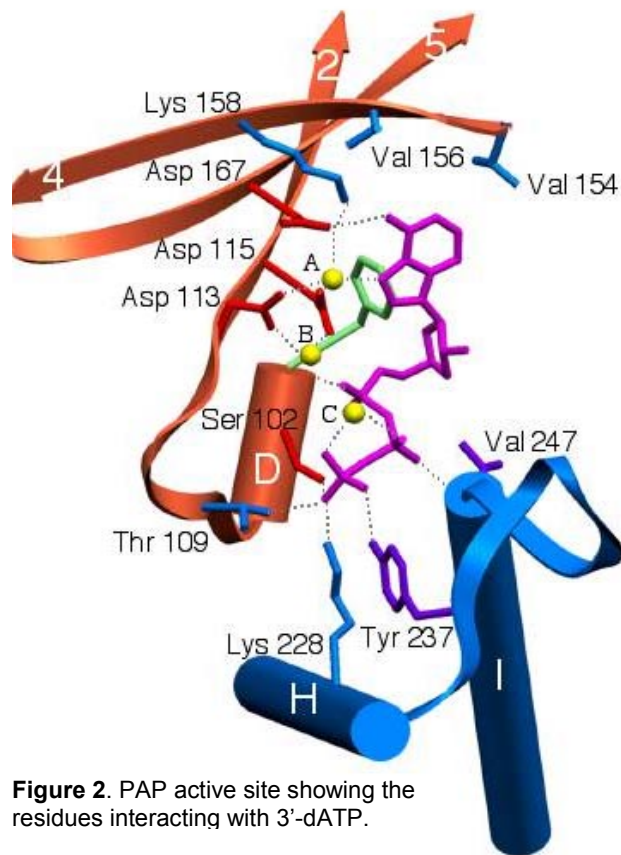
**Conclusions:** This study of bovine PAP complexed to 3'-dATP provides the first structure of any component of the mammalian polyadenylation machinery and the first example of a template-independent polymerase. In the active site, conserved, catalytically important amino acids contact the nucleotide. The three aspartates that are conserved in this family of terminal nucleotidyl transferases, and amongst other families of DNA and RNA polymerases, ligate two metals. One of these metals contacts the N7 position of the adenine ring, and could play a role in the selective incorporation of adenine. A third metal ligates three non-esterified oxygens of the triphosphate tail. This crystal structure will guide additional studies of PAP complexed to poly(A) RNA and proteins of the polyadenylation machinery that are known to interact with the polymerase, such as CPSF and PABP2.

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**References:** Martin, G., Keller W., and Doublé S. (2000), Crystal Structure of Mammalian Poly(A) Polymerase, EMBO J., 19:4193-4203.



**Figure 1.** Ribbon diagram of the bovine PAP complex with 3'-dATP.



**Figure 2.** PAP active site showing the residues interacting with 3'-dATP.